*trans* with a 5 thymidines (5T) sequence within the intron 8 Splice Variant (IVS8) region. 5T is responsible of an exacerbated skipping of exon 9, decreasing the functional product levels. It has been demonstrated that 5T phenotypic expression is influenced by another adjacent polymorphic region, constituted by 9 to 13 TG repeats. In particular, 5T/TG13 combination was found only in affected subjects.
**Case Report:** We report a case regarding two sisters, aged 23 and 18 years, carriers of the F508del mutation associated with the 5T/TG13 combination. DHPLC investigation did not detect a second mutation. Both sisters present mild pulmonary symptoms started in puberty, bronchiectasis, pancreatic sufficiency and border-line chloride values at the sweat test. However, they differ because the elder patient has more evident bronchiectasis, and she also presents pansinusitis, a positive sputum culture and a slightly reduced FEV1.
**Conclusions:** The natural history of non classic CF is poorly understood. It may be asymptomatic for years but a significant lung involvement may occur, as seen in the elder sister. This finding suggests that a prevention therapy could be necessary also in mild, non classic CF and an early diagnosis may prevent the organ deterioration, as in the younger sister. Early diagnosis may be supported by TG repeats testing in individuals carrying the 5T variant; in fact, our report confirms that the presence of 5T allele, *in trans* with a severe CFTR mutation, is associated with non classic CF and that TG13 variant acts as a real mild mutation, enhancing the 5T penetrance and determining the onset of a mild symptomatology in all patients.

## P13 ATYPICAL CYSTIC FIBROSIS ASSOCIATED WITH COMPLEX ALLELE: DIAGNOSTIC AND MANAGEMENT DILEMMAS

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**Introduction:** In recent years, an increasing number of subjects bearing "atypical" forms of cystic fibrosis (CF) associated with normal sweat chloride concentrations has been described.
**Objective:** We report a case of an atypical form of CF observed in two sisters with F508del/G576A + R668C complex CFTR genotype.
**Methods:** These patients were first examined for a panel of 56 CF mutations with Inno-Lipa reverse dot blot procedure (Innogenetics Belgium) and later with DGGE of all exons of the CFTR gene using primers and the conditions described elsewhere (Fanen P, et al. 1992. *Genomics* 13, 770–776; Costes B, et al. 1993. *Hum Mol Genet* 2, 1209–1213) followed by sequencing analysis to characterize the mutations.
**Case:** A 6-year-old female with recurrent episodes of asthma exacerbation came to the Southern Italy referral CF Centre in October 2006. She did not present symptoms of pancreatic insufficiency. However, she was hetereozygous for F508del with a normal sweat chloride test (38 mEq/L). After scanning the whole CFTR gene we individuated other mutations: G576A (exon 12) and R668C (exon 13). On the basis of these results, her sister (5-year-old) affected by recurrent episodes of upper airway infections was also examined. She showed the same CFTR genotype of her sister, her sweat chloride values were also normal (31 mEq/L).
**Discussion and Conclusion:** The G576A, originally listed as a neutral polymorphism in CF Genetic Analysis Consortium (www.genet.sikkids.on ca./cftr/) is a missense mutation that causes CFTR exon 12 skipping (Pagani F, et al. 2003. *Hum Mol Genet* 12, 1111–1120). More recently, studies documented this mutation in adults with classic forms of CF (Bienvenu T, et al. 1997.*Ann Genet* 40, 5–9) and in patients who have evidence of a clinical disease only in a subgroup of the organ systems (Ravnik-Glavac M, et al. 2000. *Pflugers Arch* 439, 53–55; Pignatti PF, 1995. *Mol Genet* 4, 635–639). These non classic CF forms, including late-onset pulmonary disease, bronchiectasis, congenital bilateral absence of vas deferens or idiopathic pancreatitis were associated with the presence of the G576A allele combined with the R668C polymorphism. Our findings suggest that our patients have the same described genetic arrangement but a longer period of follow-up is needed to get definitive conclusions. How can we manage these cases in childhood?

## P14 FROM "EVOCATIVE" SYMPTOMS TO GENOTYPE DELTAF508/E831X

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A 26-year-old subject with azoospermia was referred to our Center. The genetic test at the first level of screening showed heterozygosity for F508del.
From the history, symptoms suggestive of CF were present (three episodes of serious dehydration, bronchopulmonary pathology, easy to become tired) therefore the sweat test was performed and resulted pathological ($Cl^-$ 92/94), leading to a diagnosis of CF.
In order to identify the second mutation the analysis of the entire coding sequence of the CFTR gene was performed and we identified the mutation E831X, a G to T transition at position 2623 in exon 14a. This mutation has been reported in Cystic Fibrosis Database two cases (Ferec et al 1992).
General conditions of our patient are good, good nutritional status, PS and mild lung disease.
Because exon 14a is the subject of alternative splicing (Hull et al., 1994) and the mutation E831X is at the first base of this exon we suppose that the mutation caused a new site of splicing and increase its alternative splicing. Such a by-pass of a premature termination codon has been shown to ameliorate the disease phenotype in other clinical conditions (Morisaki et al., 1993; Ginjaar et al., 2000; Su et al., 2000). Accurate history, sweat test and mutation analysis of CFTR gene have permitted to refine the diagnosis of CF in an azoospermic adult subject.

## P15 A QUALITATIVE CHARACTERIZATION OF THE CFTR GENE BY mRNA ANALYSIS

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A significant percentage of CF alleles (ranging from 4 to 10%) remain unidentified in most population, even after extensive studies of the *CFTR* gene by PCR based procedure. At present time, CFTR mRNA analysis represents more a research tool for the identification of unknown molecular defects of the CFTR gene in specific rare cases than a routine approach to complete a diagnostic procedure. In particular mRNA analysis may allow researchers to establish the pathogenic role of sequence variations not yet defined like specific splicing defects. The results of our screening for CFTR gene at DNA level, by DHPLC and MLPA analyses showed a mutation detection rate of 94.4%. After this analysis, in our patients, 81 alleles (6%) were still unknown. Aim of this work was to evaluate the role of the CFTR analysis at mRNA level as a diagnostic method for the characterization of molecular defects in patients affected by a classic form of CF, who still had one or two unidentified alleles after an extensive analysis at DNA level. RNA was extracted with TRIzol reagent, from nasal epithelial cells and collected using cyto-brush from 7 CF patients and 3 non-CF controls. First strand cDNA was synthesized using hexanucleotide primers and high capacity cDNA Archive kit. The cDNA was amplified in six overlapping fragments spanning the entire gene and then visualized on agarose gel for identifying large deletion/insertion and the product of possible alternatives splicing; each fragment was next sequenced.
Disease-related mutations, not detected by DHPLC technique, were identified in two patients; mRNA analysis performed on two other related patients with a deletion of exon 2 at DNA level showed two novel transcription products carrying a deletion of exon 2–3 and an insertion of intron sequence of about 80bp near exon 6b, respectively. Two patients had low level of mRNA product and have to be analyzed by quantitative technique. One patient showed a normal profile. In conclusion our data suggest that the defects at RNA level could explain the pathogenic role of abnormal mRNA products in Cystic Fibrosis onset.

## P16 IMPROVEMENT OF MOLECULAR TECHNOLOGIES AND PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS

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**Introduction:** More than 1200 mutations and 200 polymorphisms have been described for CFTR gene. Prenatal Diagnosis of Cystic Fibrosis (CF) must be proposed to couples in which both partners are carriers of CFTR mutations and thus the fetus is at one in four (1/4) risk of being affected.
Since 1989 the Centro di Riferimento Regionale Toscano per la Diagnosi Genetica di Fibrosi Cistica has performed the characterization of CF patients together with prenatal diagnosis for at-risk couples. The steps to be followed with prenatal diagnosis will be as follows:

- Pre-test genetic counselling: to be performed before invasive procedures (amniocentesis or CVS) in order to advise the couples about the risk of prenatal sampling and the accuracy of the genetic test for the diagnosis of CF.
- Post-test genetic counselling: to be performed in order to illustrate the results obtained from the genetic test.

The at-risk couples are followed by a team composed by physicians, genetic counsellor and other specialists.
**Methods:** Prenatal specimen types include both amniocytes and chorionic villi. On the fetus' DNA known mutations are detected by direct sequencing or OLA-PCR; when mutations causing familial CF are unknown, CFTR gene is investigated